# Populations of Individual Species of Cellulolytic Bacteria in the Rumen of Lactating Cows Fed Different Diets

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## Introduction

Because the ruminal microflora are the key link between the ruminant animal and its diet, the species composition of the microflora are expected to have a great impact on the availability of VFAs and microbial cells used by the animal for energy and protein synthesis. In the case of forage-fed animals, the cellulolytic population would be expected to be major components of the microflora. *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* are three of the most abundant cellulolytic bacteria in the rumen. All three species are able to rapidly degrade crystalline cellulose, but these species differ in their growth yields and their fermentation endproducts, and thus the relative populations of these species should affect nutrient availability. Advances in the development of oligonucleotide probes specific for 16S ribosomal RNAs of individual microbial species has permitted quantification of individual species in their natural environment. This study describes the application of such probes to the assessment of population sizes of the three predominant cellulolytic species in the rumina of cows fed diets based on alfalfa silage or corn silage, at two different forage levels.

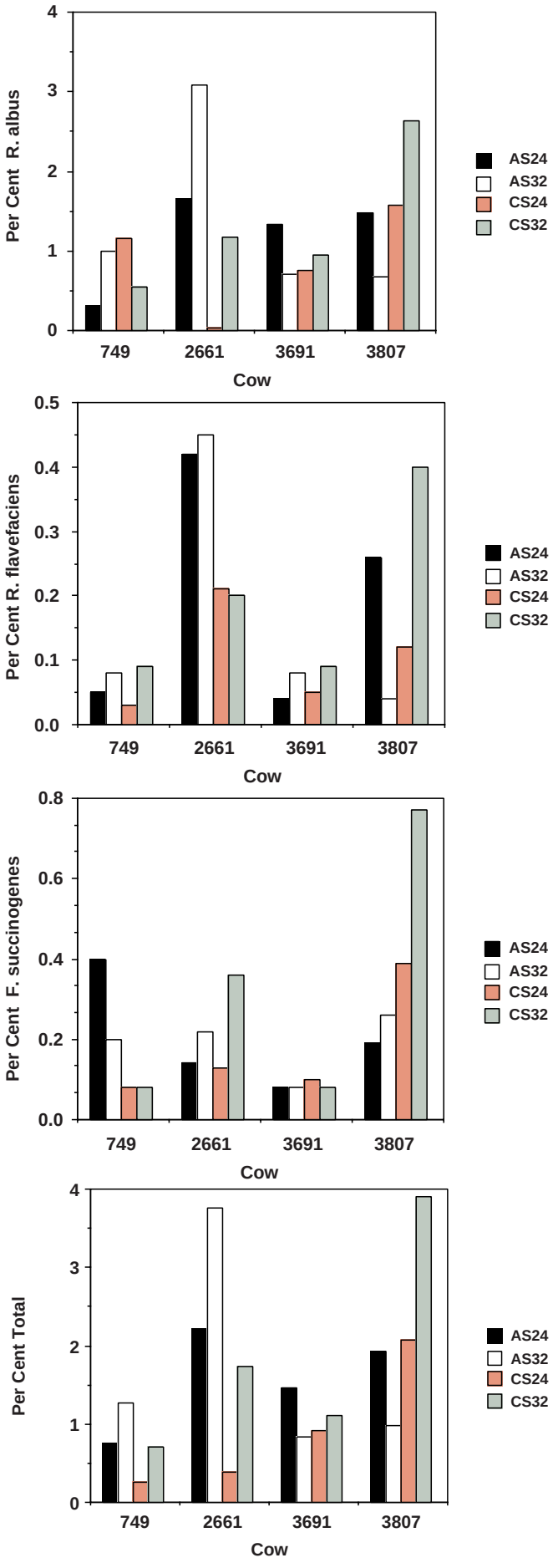
## Material and Methods

Four ruminally-fistulated, multiparous Holstein cows were fed, in a Latin square design, diets differing in source (alfalfa silage or corn silage) and amount (24% or 32% NDF, determined after treatment with a-amylase) of fiber. Diets were offered at 12h intervals as total mixed rations in amounts that assured ad libitum intake. After adaptation to diet for 19 days, 13 samples were collected from each rumen over a 4-day period. The rumen contents were separated by squeezing through cheesecloth, and the strained ruminal fluid (SRF) was used for measurement of pH and VFAs , and as inocula for in vitro digestion kinetic experiments; the data has been reported previously (Weimer et al. 1997). SRF and squeezed ruminal solids were stored at -70 $^{o}$C for isolation of microbial RNA.

For each cow-diet combination, RNA analyses were performed only with the five samples collected 3 h post-feed during the week of sampling. Thawed SRF (25 mL) was combined with thawed squeezed solids (25 g), and mixed in a chilled Waring blender. Microbial cells were recovered by centrifugation at 500 x g, and the solids washed with chilled saline and re-centrifuged. The second supernate was combined with the first, filtered through glass wool, and the cells were pelleted and stored at -70 $^{o}$C. Methods for extraction of RNA from cells, and for hybridization with digoxigenin-labeled oligonucleotide probes, and quantitation of hybridized RNA have been described previously (Shi et al. 1997). Probes used for hybridization were: S-Ss-F.s.suc-0207-a-A-21 for *F. succinogenes*, S-S-R.fla-1176-a-A-17 for *R. flavefaciens*, and S-S-R.alb-0196-a-A-18 for *R. albus*. The amounts of RNA on each densitometer image were determined from standard curves prepared with purified RNA from *F. succinogenes* S85, *R. flavefaciens* B34b, or *R. albus* 7. The corrected RNA values were normalized to total eubacterial RNA in the samples, determined from separate hybridizations using the eubacterial domain-specific probe S-D-Bact-0338-a-A-18 and commercial *Escherichia coli* RNA as standard.

## Results and Discussion

The relative population sizes of the three predominant cellulolytic species for the sixteen cow-diet combinations are shown in Fig. 1. Total RNA from the three species represented 0.3- 3.9 per cent of the total bacterial RNA, similar to the proportion of cellulolytic bacteria in the total bacterial population estimated by culture procedures (Van Gylswyk 1970, Dehority et al. 1989). *R. albus*-specific RNA was much more abundant than was that of either *R. flavefaciens* or *F. succinogenes*.




Comparison of the populations across cows is complicated by the known variation in response of different strains to species-specific probes. Moreover, the high variability of the method (CV=39.7%, averaged across 48 cow x diet x bacterial species combinations) makes comparison difficult even within diets. In general, the populations of individual species did not show obvious patterns with diet that were consistent across cows. Nevertheless, some trends are apparent. In most cases, the three cellulolytic species represented a greater fraction of the total bacterial population in the higher-fiber diets than in the lower-fiber diets, in accord with the notion that cellulose digestion in the rumen is limited by substrate (i.e., increasing cellulose concentration will increase the cellulolytic population). In cows 749 and 2661, the depressed levels of *R. albus* in the CS24 diet may have been due to the 3h-postfeed pH values (5.46 and 5.18, respectively), which were lower than those observed on other diets. This observation is in accord with the known sensitivity of ruminal cellulolytic bacteria to low pH.

## Conclusions

Despite their functional importance in fiber digestion, the three predominant species of ruminal cellulolytic bacteria represent only a small fraction of the total bacterial population across a variety of cow-diet combinations. The RNA probe method, while powerful and sensitive, had limited quantitative reproducibility that made it difficult to detect changes in populations resulting from changes in diets. Changes in the populations of the three predominant cellulolytic species, when observed, differed among cows, suggesting that each cow maintains unique assemblages of ruminal microbial strains.

*Figure 1. Populations of three predominant species of ruminal cellulolytic bacteria, and their sums, in four cows on four diets, determined using oligonucleotide probes, and expressed as a fraction of total bacterial RNA. Results are mean values of 3-5 samples collected 3h-postfeeding during the week of sampling. Note differences in the scale of each ordinate.*

## References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